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COMPLETE SPECIFICATION

Title of Invention:

Amplification of nucleic acids and detection of a new non-A, non-B, non-C, non-D, non-E, hepatitis virus

Name, address and nationality of
applicant(s) as in international
application form:
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Amplification of nucleic acids and detection of a novel hepatitis Non-A/ Non-B/Non-C/Non-D/Non-E virus

Subject matter of the invention is a reagent for the amplification of nucleic acids of a Non-A/Non-B/Non-C/Non-D/Non-E (N-ABCDE) hepatitis-associated virus and methods for its detection with the aid of this reagent.

In addition to hepatitis A virus (HAV) and hepatitis B virus (HBV) which have been known and are characterized, several other viruses have recently been characterized which are associated with hepatitis, but form independent virus groups. These groups are commonly characterized in such a manner that the group names are associated with consecutive capitalized letters. Each newly found hepatitis-associated virus is distinguished from the preceding virus in that it does not belong in the group of preferably known viruses. This is why the hepatitis C virus (HCV) had also been referred to as Non-A/Non-B hepatitis virus. The present invention refers to a virus which can obviously not be associated with the groups of HAV, HBV, HCV, HDV and HEV viruses.

WO 94/18217 also describes a virus which is not associated with one of the five above-listed groups. The nucleotide sequences mentioned in the present invention bear, however, no similarity to the sequences described in WO 94/18217.

WO 95/21922 also describes a hepatitis reagent which is not associated with any of the above-listed five groups.

In the following text, the group of hepatitis-associated viruses as defined in the present invention is referred to as HGV. Presently available information on HGV suggests that this virus belongs to the flaviviridiae family.

Subject matter of the invention is a reagent for the amplification of HGV-specific nucleotide sequences wherein HGV is defined as a virus whose genome consists of RNA with a nucleotide sequence at its 5'-end which is to at least 80%, preferably 90% homologous to the

nucleotides of SEQ.ID.NO. 1; it comprises two HGV-specific primers each with an extendable end where one primer contains a sequence of 15 to 30 bases which are to more than 80%, preferably at least 90% complementary to successive bases of the nucleotides of SEQ.ID.NO. 1; and where the other primer contains 15 to 30 bases which are up to more than 80%, preferably to at least 90% homologous to successive bases of the nucleotides of SEQ.ID.NO. 1. The extendable ends of the primer are selected such that when the primers are extended, each primer is able to hybridize with the extension product of the respective other primer, and the respective extension products can be used as templates for the extension of the respective other primer.

Amplification as understood in the invention is a method for the preparation of numerous copies of a base sequence. This can be accomplished with known methods such as the polymerase chain reaction as described in US-A-4,683,202.

A primer is understood to be a molecule which has a number of nucleobases attached to its backbone. A backbone is a polymer framework. Particularly well known is the sugar phosphate backbone of nucleic acids such as DNA and RNA. Heterocyclic compounds which are able to form hydrogen bonds with complimentary heterocycles are covalently bound to these backbones. Commonly known are the naturally occurring bases adenine, guanine, cytosine, thymine, and uracil. However, there are other bases which also occur naturally. The sequence of these bases is selected to be complimentary to more than 90% to successive bases of the nucleotide sequence to be amplified. Each of these molecules has at least one extendable end. Extension is in particular understood the enzymatically catalyzed addition of base units with the aid of mononucleoside triphosphates or oligonucleotides. When an extension is accomplished with mononucleoside triphosphate units, the preferred enzyme is DNA polymerase. The nucleic acid which contains the nucleotide sequences to be amplified serves as a template for the specific incorporation of bases. The template sequence determines the sequence of the bases which are attached to the primer.

It is expedient to use molecules with 15 to 30 bases as primers as their chemical synthesis is relatively easy to accomplish.

When a DNA polymerase is used, the 3'-end is preferably used as the extendable end. A reverse transcriptase (RNA-dependent DNA polymerase) is preferably used when the reagent

for amplifying nucleotide sequences is to be taken directly from the genomic RNA of the HGV. Since the reverse transcriptase also has DNA-dependent DNA polymerase activity, a multitude of DNAs is first formed from the so-obtained cDNA. After formation of DNA, it is also possible to use another DNA-dependent DNA polymerase, e.g. from E. coli or Thermus aquaticus.

Methods for amplifying nucleotide sequences according to the PCR principle using reverse transcriptases (RT-PCR) are known, for example, from US-A-5,310,652 or WO 91/09944. For a definition of the reaction conditions please refer to these patent applications which are to be considered part of this document.

These two primers which are complimentary to different strands of the nucleotide sequence are used to form a nucleic acid whose length is determined by the two opposing ends of the two primers which are preferably not extendable. Principally, the length of these nucleic acids which can also be referred to as amplificates can include the entire region of SEQ.ID.NO. 1, i.e. up to 348 bases in length. A preferred length of the amplificates is greater than 100 bases. In a particularly preferred manner, the length of the amplificates ranges between 150 and 200 nucleotides (nt). In addition to the sequences of the primers, the amplificate also contains a region that was newly formed as a result of the incorporation of mononucleoside triphosphates or polynucleotides.

Preferably, the primers in accordance with the invention do not hybridize with members of other hepatitis-associated virus groups, particularly not with hepatitis A, B, C, D, or E viruses. Moreover, it is preferred that they do not hybridize with other nucleic acids that are found in human blood.

Preferred primers are those that are completely complimentary or homologous to a partial sequence of SEQ.ID.NO. 1. Experience has shown, however, that in some cases it is possible to select the few nucleotides such that they have a mismatch with respect to the sequence of SEQ.ID.NO. 1. However, this mismatch is preferably not located at 3'-end of the primer.

An HGV specific nucleotide sequence is understood to be a sequence which can be found in HGV but not in other viruses and eucaryotic cells.

An HGV-subtype-specific nucleotide sequence is understood to be an HGV-specific sequence which cannot be found in all HGV subtypes. An HGV subtype is meant to be a virus which has all the phenotypical properties of HGV whose nucleotide sequence, however, is different from the other HGV. This deviation amounts to at least 10% and is preferably located within nucleotides 64-348 of SEQ. ID No. 1. Inverstigators found that the HGV subtypes differ from each other, particularly in nucleotide regions 74-92, 186-223, 255-283, and 303-306, and more particularly differetn from SEQ. ID. No 1. In a subtype-specific detection, at least one of the primers or even the probe are selected such that the sequence covers one of these regions either completely or partially.

Assuming that SEQ. ID No. 1 is a first subtype of HGV, then subtypes 2a and 2b can be defined based on the alignement in Fig. 1. Fig. 1 shows only nucleotides where the sequences differ from the sequence in SEQ. ID. No. 1.

In subtype-specific detection procedures, it is possible to select one primer HGV-subtype specific and another one HGV subtype-unspecific.

Fig. 1 shows HGV nucleotides from different subjects which correspond to nucleotides 64-348 of SEQ. ID No. 1. It can be seen that according to the above given definition the first HGV (Sa 1134 and Sa 1172) belong to a first subtype (2a) while the remaining HGV are part of a subtype of its own (2b). Note the presence of a deletion in position 255 as compared to SEQ. ID. No. 1. Fig. 1 also shows the preserved and variable regions.

HGV-subtype unspecific sequences are HGV-specific sequences which can hybridize with all known HGV subtypes. They include in particular the preserved regions of SEQ. ID. No. 1, preferably between 92 and 186 , 223 and 255 as well as 315 and 348.

HGV detection is understood to be a method of determining the presence of HGV in a sample. A preferred sample is a blood sample. A method of determining an HGV subtype can either be a method of determining a subtype or HGV per se.

In addition of HGV-specific nucleotide sequences, the primer of the invention can also contain nucleotide sequences which are neither HGV-specific nor able to hybridize with nucleic acid

commonly found in a sample. They are preferably found at the non-extendable end of the primer. These additional nucleotide sequences can be used to capture the amplificates.

It is also possible to further modify the primers, e.g. by attaching a detectable or immobilizable group. Such groups include, for example, directly or indirectly detectable groups, e.g. radioactive, stained or fluorescent groups. Indirectly detectable groups include immunologically or enzymatically active compounds, such as haptens or enzymes. They are detected in a subsequent reaction or a reaction sequence. Haptens as described in EP-B-0 324 474 (digoxigenin) are particularly preferred. Biotin has also proven well as an immobilizable group as it immobilizes well on surfaces that are coated with avidin or streptavidin. This allows either capturing the amplificates on a solid phase and removing sample constituents or the detection of substances that may interfere with the amplification reaction.

Particularly preferred primers are those which contain a sequence of six successive bases contained in the sequences of SEQ.ID.NOS. 2 or 3 or 5 to 20 or a sequence which is complimentary thereto. Particularly preferred are primers as described in SEQ.ID.NOS. 2 or 3 or 5 - 20.

Nucleotides Y and R which may appear in the SEQ. ID are meant to indicate that the described primers are a mixture corresponding to the meaning of the letters Y and R.

Another subject matter of the invention is a reagent kit for the specific detection of HGV comprising a reagent according to one of the claims 1 to 4 and a probe containing a base sequence located within the extension product formed in the amplification reaction, i.e. between the extended ends of the primers. A probe is understood to be a molecule which has a number of nucleobases at a backbone as does a primer. However, the probe must not necessarily have an extendable end. A probe is characterized in that it contains a recognizable group. Such a recognizable group can be a detectable group or an immobilized or immobilizable group. A definition of such groups was already given under the section that mentions primers. In a particularly preferred manner, the probe has an immobilizable residue, i.e. a residue which can be bound to a solid phase or a solid body in a subsequent or

simultaneous reaction. If the immobilizable group is a biotin residue, the solid phase can be the streptavidin or avidin-coated surface of a microtiter plate or a tube.

In a preferred manner, the reagent kit contains the primers and the probes in separate containers. Moreover, the kit can contain other components that are necessary or support the amplification and the detection, such as a reverse transcriptase, mononucleoside triphosphates, which are preferably labeled, so as to be detectable; then, a solid phase for capturing the hybrid consisting of probes and amplificates, and buffering substances.

Another subject matter of the invention is, hence, a method for the detection of HGV in a sample, characterized by forming cDNA from a part of the HGV RNA and the amplification of a cDNA with the aid of a reagent according to one of the claims 1 to 4; by bringing the amplification products into contact with a probe containing a base sequence which is located between the extendable ends of the primers; and determining the formation of hybrids of probe and amplification products to demonstrate the presence of HGV in the sample.

The present invention advantageously provides a specific method for the detection of HGV which is still reliable when assaying samples of patients who are positive for either one of the HGV subtypes and where HCV has not been shown. Moreover, the method of the invention allows the detection of HGV with a high degree of sensitivity. The specificity in a Southern Blot is also high.

Further, if a reliable diagnosis is desired, it is possible to combine the detection according to the method of the invention with an RNA detection at another site of the HGV genome; in particular, with an RT-PCR in a region which codes for Non-structural HGV proteins. The sequence of the non-structural regions can be determined according to the method described in EP-B-0 318 216. In order to accomplish this, the primers are extended in 3'-direction of the genome starting with the sequence of SEQ.ID.NO. 1; then the sequence of the extension product is determined. This sequence is then used to obtain a primer which is used in yet another extension reaction in 3'-direction of the genome, and so forth. The successful accomplishment of at least one of the two amplification reactions then confirms the presence of HGV.

The following examples explain the invention in greater detail:

Example 1

HGV-specific detection

Reagents

- QIAamp, HCV kit (QIAgen Cat. No. 29504)
- M-MuLV-reverse transcriptase (Boehringer Mannheim, 1062603)
- PCR nucleotide mix (Boehringer Mannheim, 1581295)
- Hexamer random mixture (Boehringer Mannheim, 1277081)
- RNase inhibitor (Boehringer Mannheim, 799017)
- Sterilized bidistilled water
- Primer 1 (SEQ.ID.NO. 2)
- Primer 2 (SEQ.ID.NO. 3)
- Biotinylated probe nucleic acid (SEQ.ID.NO. 4), obtained via amino linker (Applied Biosystems Inc.)
- PCR ELISA (DIG labeling kit, Boehringer Mannheim, 1636120) (or single reagents PCR-DIG labeling mix (Boehringer Mannheim, 1585550) and Taq-DNA polymerase (Boehringer Mannheim, 1146165))
- Enzymun-Test® DNA detection (Boehringer Mannheim, 1447777)

General recommendations

The entire procedure should be carried out at three different work places, namely one for RNA isolation, one for the preparation of reverse transcriptions, the amplification reactions and their performance, and one for the detection of the amplification products. Each work place should

be provided with separate sets of pipettes. In order to avoid contamination use sealed aerosol-tight pipette tips or special PCR pipettes. Prepare the reagents fresh every day and decontaminate all pipettes on a regular basis. Also use new gloves at each work place.

Sample preparation

The QIAamp HCV kit was used to prepare total RNA from human sera according to the manufacturer's instruction. It is also possible to employ other RNA preparation methods, such as acid phenol extraction according to Chomczynski or the use of guanidinium isothiocyanate.

cDNA synthesis (reverse transcription)

The preparation of reverse transcription and amplification mixtures is carried out at a separate work place using an individual set of pipettes. The reagents used in the reaction are listed in the following table.

Table 1

Reagents	Stock solution	Volume per reaction	Final concentration in RT buffer
5 x reverse transcriptase buffer	5 x	4 µl	1 x
PCR dNTP mix	10 mM each	0.2 µl	100 µM
Random hexamers	20 µM	0.5 µl	200 nM
RNase inhibitor	40 U	0.5 µl	1 U
H_2O	-	3.8 µl	-
m-MuLV reverse transcriptase	20 U/µl	1 µl	20 U/reaction
RNA		10 µl/reaction	

10 µl of the RT mix are pipetted into the PCR reaction vessels. Subsequently, 10 µl of RNA solution are added. This mixture is briefly treated in a Vortex mixture, centrifuged and then incubated for 10 minutes at room temperature, for 30 minutes at 42°C and for 5 minutes at 95°C. Then, the reaction mixture is stored at 4°C until further treatment is carried out.

Labeling reaction

The preparation of the amplification mixture is carried out at a separate work place using a separate set of pipettes. It is not necessary to pipette the amplification mixture on ice. The reagents used are listed the following table.

Table 2

Reagents	Stock solution	Volume per reaction	Final concentration
10 x PCR incubation buffer	10 x	5 µl	1 x
PCR DIG-labeling mix			
dATP	2 mM		200 µM
dCTP	2 mM		200 µM
dGTP	2 mM	5 µl of the	200 µM
dTTP	1.9 mM	DIG-labeling	190 µM
DIG-11-dUTP	0.1 mM	mix	10 µM
Primer 1 (SEQ.ID.NO. 2)	5 µM	2 µl	200 nM
Primer 2 (SEQ.ID.NO. 3)	5 µM	2 µl	200 nM
Taq DNA polymerase	5 U/µl	0.5 µl	2.5 U/reaction
H_2O		fill up until 40 µl	
cDNA solution		10 µl	

40 µl of the amplification mixture are prepared in the reaction vessels. 10 µl of the solution from the cDNA synthesis are added. The mixture is then briefly mixed in a Vortex mixer and then centrifuged. Subsequently, the reaction vessels are placed into a Perkin-Elmer thermocycler (PE9600) and 40 cycles are carried out (15 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C). Subsequently, the reaction mixture is stored at 4°C until the detection reaction is carried out. If the mixture is to be stored over an extended period of time, this should be done at - 20°C.

Detection of the amplification products

The detection of the digoxigenin-labeled amplification products is carried out with the aid of the Enzymun-Tests DNA detection (on Boehringer Mannheim ES instruments) or with the aid

of PCR ELISA tests (on Boehringer Mannheim microtiter plates,). Pipetting of the amplification products and their detection is carried out at a third work place. Prior to opening the reaction vessels, the solution is briefly centrifuged. The solutions are diluted 1:10 using the denaturing solution from one of the above-listed kits. The concentration of the capture probe is preferably 75 ng/ml in the hybridization solution.

Detailed protocols for the detection of amplification products are given in the above kits (Enzymun-Test DNA detection) for the ES system (ES 300, ES 600, ES 700) and PCR ELISA for the microtiter plate format.

Alternative primers

The primers of SEQ. ID. No. 5, 6, 7, 8, and 9 have also proven to be effective in accordance with the invention, particularly when 5/6, 5/7, and 2/9 are combined.

With primer combination 2/8, it was possible to detect 3 different genotypes; this is hence a HGV-subtype-specific detection.

Example 2

HGV subtyping

Except for the primers, all conditions listed in Example 1 apply.

Certain pairs (Seq. ID. No./SEQ. ID. No.) are used as primers; the results are those given in table 1. Values (absorbances) exceeding 100 were considered as positive test results. This shows that it is possible to selectively detect individual subtypes by selecting certain primer pairs.

Table 3

	Samples	100-43	100-44	701-1134	701-1172	701-1119	701-1151	701-1153
Primer combination	**Genotype**	**1**	**1**	**2a**	**2a**	**2b**	**2b**	**neg.**
2/8	**alle**	4622	4717	3976	4459	4104	4317	35
2/12	**1**	4758	4678	32	53	847	32	15
2/13	**2a+b**	51	52	4553	4986	3938	4028	45
2/14	**1**	4607	4633	30	61	32	28	61
2/15	**2a**	55	53	4331	4729	33	35	57
2/16	**2b**	44	39	34	58	3641	3466	54
2/17	**1**	4774	4688	31	64	35	32	61
2/18	**2a+b**	73	56	4696	5016	4828	4696	74
2/19	**1+2a**	4413	4931	4727	5063	15	36	57
2/20	**2b**	45	49	31	50	698	4126	64
2/3	**1+2a**	91	4695	5491	4671	27	52	32

Values with gray shading were different from expected values; they matched, however, when the experiment was repeated under revised stringency conditions (2/12 negative; 2/20 positive).

Table four is a listing of the primer sequences and their individual HGV specificities.

Table 4

SEQ.ID.NO.	Recognition of genotypes	Sequence (5'-3')
2	alle	CGG CCA AAA GGT GGT GGA TG
8	alle	AAC ACC TGT GGA CCG TGC G
10	1	CAA TGA CTC GGC GCC GAC
11	2a + b	TGA TGG CCC YGC GCC RAA
12	1	AGA GGA ATC TTA ACC TTC TC
13	2a + b	AGA GGG ACC GTA GCC TCC C
14	1	GGT CTC GCC GCA GGC ACA
15	2a	CGT TCT CGC CAC GGG CAT T
16	2b	CTT TCY CTC CRT AAG CGC G
17	1	TCG GGC CCT TAT TCA CAC C
18	2a + b	CCG GGY CCT TAT TAC ACC
19	1 + 2a	TAA CGA CGA GCC TGA CGT C
20	2b	TAA CGG CGT GCC TAG CGC C
3	1 + 2a	CGA CGA GCC TGA CGT CGG G

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Boehringer Mannheim GmbH

(B) STREET: Sandhoferstr. 116

(C) CITY: Mannheim

(E) COUNTRY: DE

(F) POSTAL CODE (ZIP): 68305

(G) TELEPHONE: 0621 759 4348

(H) TELEFAX: 0621 759 4457

(ii) TITLE OF INVENTION: Amplification of nucleic acids and detection of a novel hepatitis Non-A/Non-B/Non-C/Non-D/Non-E virus

(iii) NUMBER OF SEQUENCES: 20

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 348 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(viii) POSITION IN GENOME:

(A) CHROMOSOME/SEGMENT: nichttranslatierte Region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACGTGGGGGA GTTGATCCCC CCCCCCCGGC ACTGGGTGCA AGCCCCAGAA ACCGACGCCT 60

ATCTAAGTAG ACGCAATGAC TCGGCGCCGA CTCGGCGACC GGCCAAAAGG TGGTGGATGG 120

GTGATGACAG GGTTGGTAGG TCGTAAATCC CGGTCACCTT GGTAGCCACT ATAGGTGGGT 180

CTTAAGAGAA GGTTAAGATT CCTCTTGTGC CTGCGGCGAG ACCGCGCACG GTCCACAGGT 240

GTTGGCCCTA CCGGTGGGAA TAAGGGCCCG ACGTCAGGCT CGTCGTTAAA CCGAGCCCGT 300

TACCCACCTG GGCAAACGAC GCCCACGTAC GGTCCACGTC GCCCTTCA 348

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CGGCCAAAAG GTGGTGGATG 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGACGAGCCT GACGTCGGG 19

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION:1..19

(D) OTHER INFORMATION:/note= "G am 5'-Ende ist ueber Aminolink kovalent mit Biotin verbunden"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGTAGCCACT ATAGGTGGG 19

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCAGAAACCG ACGCCTATC 19

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTTATTCCCA CCGGTAGGGC 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CAAGAGAGAC ATTGAAGGGC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AACACCTGTG GACCGTGCG 19

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCACTGGTCC TTGTCAACTC 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CAATGACTCG GCGCCGAC 18

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TGATGGCCCY GCGCCRAA 18

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

AGAGGAATCT TAACCTTCTC 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AGAGGGACCG TAGCCTCCC 19

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGTCTCGCCG CAGGCACA 18

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CGTTCTCGCC ACGGGCATT 19

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CTTTCYCTCC RTAAGCGCG 19

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TCGGGCCCTT ATTCACACC 19

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCGGGYCCTT ATTACACC 18

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TAACGACGAG CCTGACGTC 19

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Oligodesoxyribonukleotid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TAACGGCGTG CCTAGCGCC 19

Claims

329902

1. Reagent for the amplification of HGV-specific nucleotide sequences, wherein HGV is defined as a virus whose genome consists of RNA containing at its 5'-end a nucleotide sequence which is to at least 80% homologous to SEQ.ID.NO. 1 (as defined herein), containing two HGV-specific primers each having an extendable end, where one primer contains a sequence of 15 to 30 bases, said sequence being more than 80% complimentary to successive bases of SEQ.ID.NO. 1 (as defined herein), and where the other primer contains a sequence of 15 to 30 bases which are to more than 80% homologous to successive bases of SEQ.ID.NO. 1 (as defined herein), wherein the extendable ends of the primers are selected such that when the primers are extended, each primer can hybridize with the extension product of the respective other primer, and the respective extension products serve as templates for the extension of the respective other primer.

2. Reagent according to claim 1, characterized in that at least one of the primers contains a sequence of 6 successive bases contained in the sequence SEQ.ID.NO. 1 (as defined herein) or a sequence complimentary thereto.

3. Reagent according to one of the claims 1 or 2, characterized in that at least one of the primers contains a sequence of SEQ.ID.NO. 2 or 3 or 5 – 20 (as defined herein).

4. Reagent according to one of the aforementioned claims, characterized in that at least one of the primers is detectably labeled.

5. Reagent according to one of the aforementioned claims, characterized in that one or both of the primers are HGV-subtype-unspecific.

6. Reagent according to one of the claims 1 to 4, characterized in that one or both of the primers are HGV-subtype-specific.

7. Reagent according to one of the claims 5 or 6, characterized in that one primer is HGV-subtype-unspecific and another is HGV-subtype-specific.

8. Reagent kit for the specific detection of HGV comprising a reagent according to one of the aforementioned claims and a probe containing a base sequence which is located within the formed extension product between the extended ends of the primers.

9. Reagent according to claim 8, characterized in that the base sequence contains at least 6 successive bases contained in the sequences of SEQ.ID.NO. 2-20 (as defined herein) or a sequence complimentary thereto.

10. Reagent kit for the specific detection of HGV comprising a reagent according to one of the claims 1 to 7 and another reagent comprising additional primers for the amplification of HGV nucleotide sequences.

11. Reagent kit according to claim 10 comprising for each pair of primers one probe for the detection of the extension product.

12. Method for the detection of HGV sample, characterized by forming cDNA from a part of the HGV-RNA and amplification of the cDNA under extension of the primers of a reagent according to one of the claims 1 to 7, while forming amplification products, bringing the amplification products into contact with a probe containing a base sequence located between the extendable ends of the primers, and observing the formation of hybrids between probe and amplification products to demonstrate the presence of HGV in the sample.

13. Method for the detection of HGV in a sample, characterized by

- forming cDNA from two or more parts of HGV-RNA and amplifying the cDNA using a reagent according to one of the claims 1 to 4 and using an additional HGV-specific primer pair,

- bringing the amplification products into contact with a probe per amplified part,

- observing the formation of hybrids between probes and amplification products and,

- evaluating the hybrid formation between the probes and the amplification products, wherein the formation of a hybrid of one amplification product confirms the presence of HGV in the sample.

14. A reagent according to claim 1 substantially as herein described or exemplified.

15. A reagent kit according to claim 8 substantially as herein described or exemplified.

16. A method according to claim 12 or 13 substantially as herein described or exemplified.

END OF CLAIMS

FIG 1

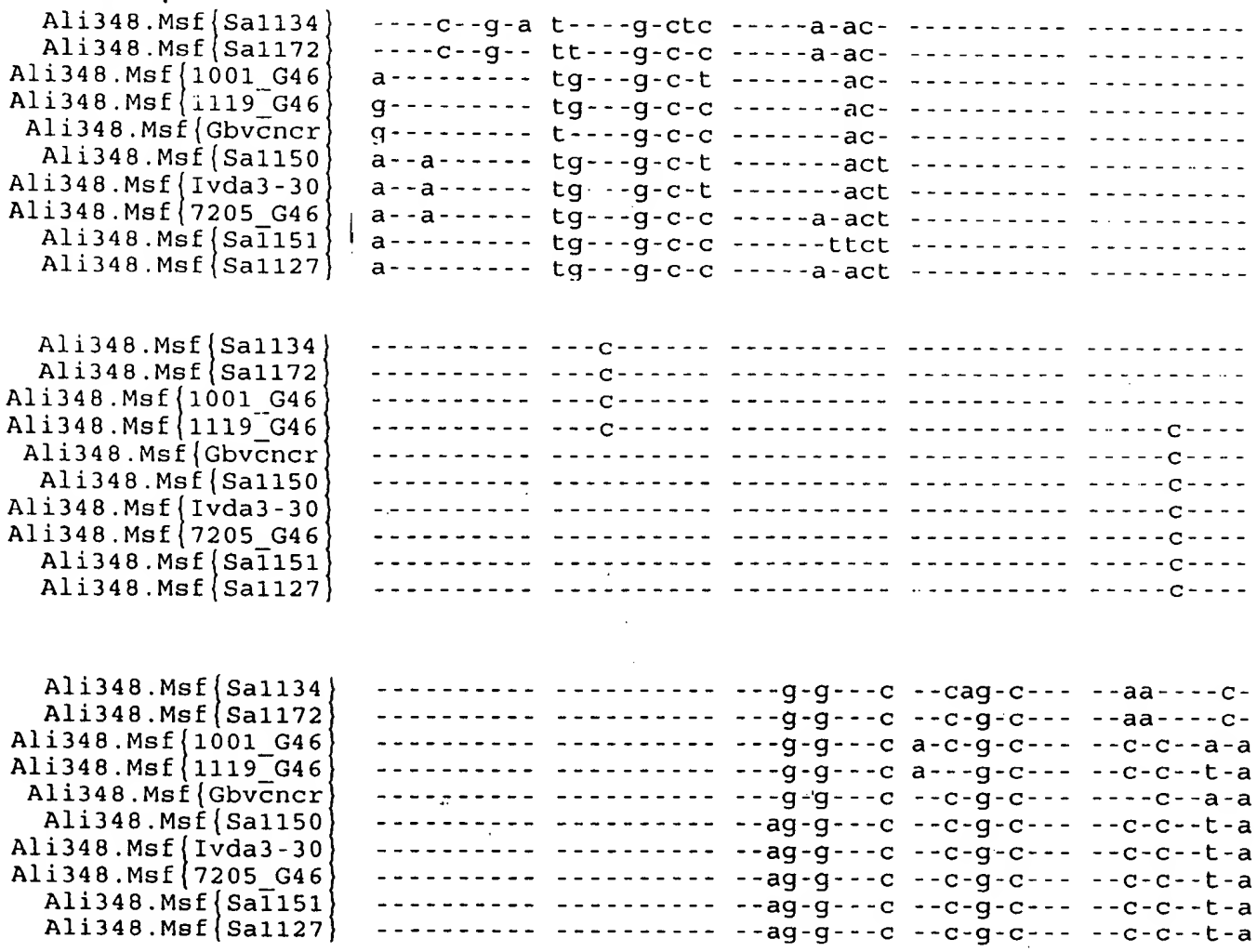
Ali348.Msf{Sa1134} ----c--g-a t----g-ctc -----a-ac- ---------- ----------
Ali348.Msf{Sa1172} ----c--g-- tt---g-c-c -----a-ac- ---------- ----------
Ali348.Msf{1001_G46} a--------- tg---g-c-t -------ac- ---------- ----------
Ali348.Msf{1119_G46} g--------- tg---g-c-c -------ac- ---------- ----------
Ali348.Msf{Gbvcncr} g--------- t----g-c-c -------ac- ---------- ----------
Ali348.Msf{Sa1150} a--a------ tg---g-c-t -------act ---------- ----------
Ali348.Msf{Ivda3-30} a--a------ tg---g-c-t -------act ---------- ----------
Ali348.Msf{7205_G46} a--a------ tg---g-c-c -----a-act ---------- ----------
Ali348.Msf{Sa1151} a--------- tg---g-c-c ------ttct ---------- ----------
Ali348.Msf{Sa1127} a--------- tg---g-c-c -----a-act ---------- ----------
Ali348.Msf{Sa1134} ---------- ---c------ ---------- ---------- ----------
Ali348.Msf{Sa1172} ---------- ---c------ ---------- ---------- ----------
Ali348.Msf{1001_G46} ---------- ---c------ ---------- ---------- ----------
Ali348.Msf{1119_G46} ---------- ---c------ ---------- ---------- -----c----
Ali348.Msf{Gbvcncr} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{Sa1150} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{Ivda3-30} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{7205_G46} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{Sa1151} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{Sa1127} ---------- ---------- ---------- ---------- -----c----
Ali348.Msf{Sa1134} ---------- ---------- ---g-g---c --cag-c--- --aa----c-
Ali348.Msf{Sa1172} ---------- ---------- ---g-g---c --c-g-c--- --aa----c-
Ali348.Msf{1001_G46} ---------- ---------- ---g-g---c a-c-g-c--- --c-c--a-a
Ali348.Msf{1119_G46} ---------- ---------- ---g-g---c a---g-c--- --c-c--t-a
Ali348.Msf{Gbvcncr} ---------- ---------- ---g-g---c --c-g-c--- ----c--a-a
Ali348.Msf{Sa1150} ---------- ---------- --ag-g---c --c-g-c--- --c-c--t-a
Ali348.Msf{Ivda3-30} ---------- ---------- --ag-g---c --c-g-c--- --c-c--t-a
Ali348.Msf{7205_G46} ---------- ---------- --ag-g---c --c-g-c--- --c-c--t-a
Ali348.Msf{Sa1151} ---------- ---------- --ag-g---c --c-g-c--- --c-c--t-a
Ali348.Msf{Sa1127} ---------- ---------- --ag-g---c --c-g-c--- --c-c--t-a

FIG 1 (Fortsetzung)

```
  Ali348.Msf{Sa1134}  t-----c-aa ---------- ---------- --t------- -.tgt-----
  Ali348.Msf{Sa1172}  t-------a- ---------- ---------- --t------- -.tgt-----
Ali348.Msf{1001_G46}  t--a-g--aa ---------- ---------- --t------- -.tgt-----
Ali348.Msf{1119_G46}  t--a-g--aa ---------- ---------- --t------- -.tgt-----
 Ali348.Msf{Gbvcncr}  t--a-ga-aa ---------- ---------- --t------- -.tgt-----
  Ali348.Msf{Sa1150}  ---a----aa ---------- ---------- ---------- -.tgt-----
Ali348.Msf{Ivda3-30}  ---a----aa ---------- ---------- ---------- -.tgt-----
Ali348.Msf{7205_G46}  -|-a----aa ---------- ---------- ---------- -.tgt-----
  Ali348.Msf{Sa1151}  t--a----aa ---------- ---------- --t------- -.tgt-----
  Ali348.Msf{Sa1127}  t--a----aa ---------- ---------- ---------- -.tgt-----

  Ali348.Msf{Sa1134}  --a------- ---------- ---------- -----a---t --c-------
  Ali348.Msf{Sa1172}  --a------- ---------- ---------- -----a---t -tc-------
Ali348.Msf{1001_G46}  --a----g-- ct----a--c ---------- ---------t -tc-------
Ali348.Msf{1119_G46}  --a----g-- at----a--c ---------- ---------t --c-------
 Ali348.Msf{Gbvcncr}  --a----g-- ct----a--c ---------- ---------- t-c-------
  Ali348.Msf{Sa1150}  -------g-- ct----a--c ---------- --a------t --c-------
Ali348.Msf{Ivda3-30}  -------g-- ct----a--c ---------- --a----c-- --c-------
Ali348.Msf{7205_G46}  -------g-- ct----a--c ---------- --a------t --c--c----
  Ali348.Msf{Sa1151}  --a----g-- ct-t--a--c ----c----- --a----c-- --c-------
  Ali348.Msf{Sa1127}  -------g-- ct----a--c ---------- --a------t --c-------

  Ali348.Msf{Sa1134}  ---------- ---------- -t-------- -----.
  Ali348.Msf{Sa1172}  ---------- --t------- -t-------- -----.
Ali348.Msf{1001_G46}  ---------- --t------- ---------- -----.
Ali348.Msf{1119_G46}  ---------- --t------- ---------- -----.
 Ali348.Msf{Gbvcncr}  ---------- ---------- ---------- -----.
  Ali348.Msf{Sa1150}  ---------- ---------c ---------- -----a
Ali348.Msf{Ivda3-30}  ---------- ---------c -t-------- -----.
Ali348.Msf{7205_G46}  ---------- --t------- ---------- -----.
  Ali348.Msf{Sa1151}  ---------- ---------c ---------- -----.
  Ali348.Msf{Sa1127}  ---------- ---------c ---------- --a--.
```